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Pre-exposure via wire-mesh partition reduces intraspecific aggression in male, wild-type Norway rats

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Abstract

*There are instances when animals are introduced and expected to live alongside unfamiliar conspecifics within zoos, laboratories and wildlife sanctuaries. These pairings of unfamiliar animals may result in stress, trauma, or even death, in addition to reduced confidence in data resulting from these subjects. For species that communicate relatedness, sex, social status, and emotional state through olfactory cues (eg pheromones), one means of counteracting aggression may involve a period of partial separation — where animals are close enough to become acquainted — while a permeable barrier maintains separation. For our study, we evaluated the use of a novel, autoclavable, wire-mesh partition to separate potential aggressors. We tested different pairs of 24 wild-type male Norway rats (*Rattus norvegicus*), previously kept in social isolation for seven days. Each control pair were merged directly into one cage, while pairs from the experimental groups underwent three pre-exposure sessions that lasted two to four days. We used continuous video recordings to assess five common threat displays: lateral threat, keep down, upright posture, chase, and clinch attack. We used two types of bedding: new (unscented) bedding and recently used bedding that conveyed scents from both merged rats. We found that rats subjected to pre-exposure demonstrated lower aggression levels across three of the five metrics (lateral threats, upright postures, and keep downs). We conclude that permeable partitions show promise as a humane mechanism to mix new individuals into pre-existing colonies. Further research may explore whether partitions could be helpful with other species that communicate social information by pheromones or direct visual inspection.*

Keywords: *animal welfare, olfaction, pre-exposure,* Rattus norvegicus*, resident-intruder aggression, social isolation*

Introduction

Intraspecific aggression between newly introduced animals is a challenge to the welfare of animals utilised by laboratory technicians, industry, conservation and animal breeders (Sanchez *et al* 1993; Marchant-Forde & Marchant-Forde 2005; Tresz & Wright 2006; Van Loo *et al* 2007; Hartmann *et al* 2009; Fawcett & Rose 2012; Tallent *et al* 2018). The presence of an unfamiliar conspecific is stressful and causes hormones such as glucocorticoids to rise in the blood and heart rate to increase. If direct interactions take place, animals may be injured or even killed as a result of fighting (Miczek & Mutschler 1996; Barnett 2009). This aggression and stress may translate into lower breeding efficiency, higher costs (including the costs of veterinary care), as well as poorer health and quality of the animals bred.

Grouping unacquainted adult individuals has long been an issue in industrial breeding where the stress of introductions has an impact on breeding costs and animal quality (Marchant-Forde & Marchant-Forde 2005; Hartmann *et al* 2009). Challenges also tend to arise when new individuals are introduced into existing groups in laboratories (Lore *et al* 1984; Krohn *et al* 2006; Tallent *et al* 2018), or when new groups are formed in, eg zoos or wildlife sanctuaries (Ruiz-Miranda *et al* 1998; Johnson 2015). The problem is particularly prominent among captive wild animals, which contrary to their domesticated or laboratorised counterparts, are likely to be far more distressed, less docile and have broader behavioural repertoires (Lockard 1968; Diamond 2002; Stryjek & Pisula 2008; Stryjek *et al* 2012; Troxell-Smith *et al* 2016). For these, and other reasons, researchers sometimes need to work with wild animals in laboratory conditions (for a review, see Stryjek *et al* 2021). Hence, there is a need to design techniques to reduce intraspecific aggression. This need is especially true if one takes into account the fact that diminished welfare and increased mortality among laboratory animals, such as rodents, impedes the quality of research conducted on those individuals (Hurst *et al* 1999; Van Loo *et al* 2002; Mumtaz *et al* 2018), or can eliminate the behaviour under study (Pisula *et al* 2006).

Standard procedures used in research laboratories often involve separating animals, either for breeding purposes or in connection with the experimental protocol. Due to the relatively short social memory in rodents, re-establishing groups (Camats-Perna & Engelmann 2017; Tzakis & Holahan 2019) may result in increased intraspecific aggression (Lore *et al* 1984; Krohn *et al* 2006). In extreme cases, it is necessary to euthanase the animals which, apart from the ethical issues, prevents such animals from being used for breeding or as subjects in subsequent experiments.

Also, such measures are contrary to the 3Rs principles of animal welfare (Replacement, Reduction, and Refinement; Russell & Burch 1959) and can increase experimentation costs.

These intraspecific aggressive behaviours are typical, adaptive, and usually related to either territorial or maternal defensive actions and/or the establishment and maintenance of social status within a group or play a dispersal function (Calhoun 1963; Moore 1999; Miczek & de Boer 2005; Barnett 2009). In social animals such as rats (Patterson-Kane *et al* 2002), pairing individuals from different groups, or introducing new individuals into existing groups, results in the need to establish a new social hierarchy, and consequently, in-fighting to gain or maintain the dominant position (Miczek & de Boer 2005). Introduced conspecifics may also lead to new aggression related to the access to resources such as food, females, and shelter (van Staaden 2011; Huntingford & Turner 2013). In the natural environment, the dominated individuals usually have the option of leaving the group or hiding from the aggressor. Whereas, in artificial breeding conditions they are deprived of these options (Blanchard *et al* 1994; Miczek & de Boer 2005; Barnett 2009), which further increases the risk of aggressive behaviour and physical harm.

However, animals continue to be merged with one another, because individual housing of social species, such as rats, is forbidden unless special ethics permits are acquired (National Research Council 2010; EU Directive 010/63/EU). The rationale for this involves the fact that among rodents, laboratory animals housed individually in the juvenile period exhibit deficient reactions to aggressive behaviours and problems with social behaviour regulation (Wongwitdecha & Marsden 1996; Von Frijtag *et al* 2002; Matsumoto *et al* 2005; Mumtaz *et al* 2018).

Given the reasons above, it is important for the welfare of social species that researchers and caretakers develop and test methods for minimising risks associated when adult animal groups are formed. Considering that familiarity is the most important factor in reducing the probability of animal aggression (Marler 1976; Huntingford & Turner 1987), it is reasonable to begin with approaches that introduce humane methods for helping the animals become acquainted with one another before being forced into a new group or pairing. For instance, in some contexts, such as industrial breeding, aggression between unacquainted individuals is sometimes reduced by installing appropriate, permeable barriers and pens (Kennedy & Broom 1994; Jensen & Yngvesson 1998). The same effect has also been observed in the presence of dominant boars with a barrier placed between them at the time when sows join existing groups (Docking *et al* 2001). The method of temporal separation of unacquainted animals while introducing them to each other ('pre-exposure') has been successfully applied to mice (*Mus musculus*; Connor & Lynds 1977) and kangaroo rats (*Dipodomys heermanni*; Thompson *et al* 1995).

Pre-exposing unfamiliar adult individuals has been advised for mice (Fawcett & Rose 2012), but has only been considered with Norway rats (*Rattus norvegicus*) on a small scale (Evans *et al* 1968).

This approach showed some promise anecdotally (Stryjek 2008) but has not been systematically tested. It seems reasonable, however, that owing to the importance of olfactory cues in rat communication (Miczek & de Boer 2005; Barnett 2009), pre-exposure may allow scents (eg pheromones) to be relayed without allowing physical contact.

The mechanism for this approach is based on the natural history of rodents. Rodents are macrosmatic species that depend on scents to recognise conspecifics, resources and to navigate (Miczek & de Boer 2005; Choleris *et al* 2009; Kiyokawa *et al* 2009, 2017; Parsons *et al* 2018, 2019). Due to rodents' poor vision, visual cues were not thought to play a significant role in social interactions, while auditory feedback (eg ultrasonic vocalisation [USV]) was thought to be limited to supportive information (Shair *et al* 1997). However, recent research (Willadsen *et al* 2014; Nakashima *et al* 2015), suggests that rats are capable of perceiving even subtle visual and auditory emotional signals from conspecifics. Under lighted conditions, they also rely on visual cues and are capable of judging *inter alia* inter-animal distance (Pellis *et al* 1996). Thus, we stop short of suggesting the mechanistic explanation is based solely on olfaction.

Indeed, research over the past five years demonstrates that multiple senses work together (eg multimodal cues) to transmit and receive inter- and intraspecific information (Lecker *et al* 2015; Munoz & Blumstein 2018). We hypothesise that separating wild, male rats using a specially designed partition inserted into a standard laboratory cage prior to a direct contact between the animals, may allow them to become acquainted with one other using multimodal cues (olfactory, auditory, visual, and [to a limited extent] tactile contact), and therefore, substantially decrease the risk of direct attacks and physical injuries. We also hypothesise that because rats mark their territory by urine scent-marking, and since intrusions of other individuals to the territory elicit strong territorial aggression (Koolhaas *et al* 2013), the rats from the Mixed group (tested on blended, soiled bedding from the territories of both rats from the observed pairs), would express a higher level of aggression than the pairs tested on clean bedding.

Materials and methods

Ethical statement

All experimental procedures were approved by the Second Local Ethics Committee for Animal Experimentation in Warsaw (Permit Number: 69/2014). All rats were cared for in accordance with the international guidelines of animal care and use for experimental procedures (2010/63/EU) and the Regulation of the Polish Minister of Agriculture and Rural Development of 10 March 2006 on laboratory animal

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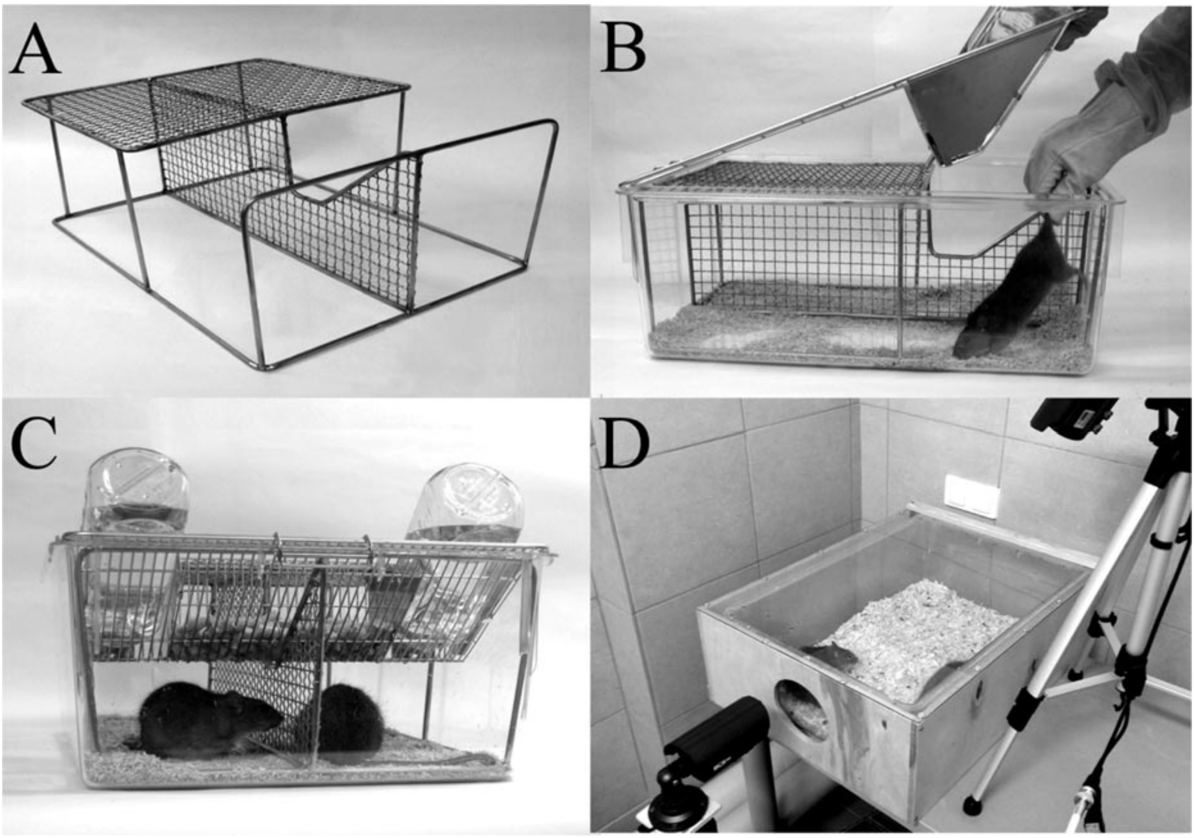
Figure 1



Images showing (A) the partition used to separate the rats housed in the same cage, (B) a rat being inserted into one of the compartments, (C) wild rats housed in the breeding cage divided by the partition and (D) the wooden case placed on top of the cage with a polycarbonate ceiling which made video recording possible.

care. Animals were carefully observed for a minimum of three days following each interaction with a conspecific and no animals were injured, as evidenced by the lack of bleeding, scars, or movement or behavioural impairments, and because no observation had to be ceased due to excessive, prolonged bouts of aggression.

Study animals

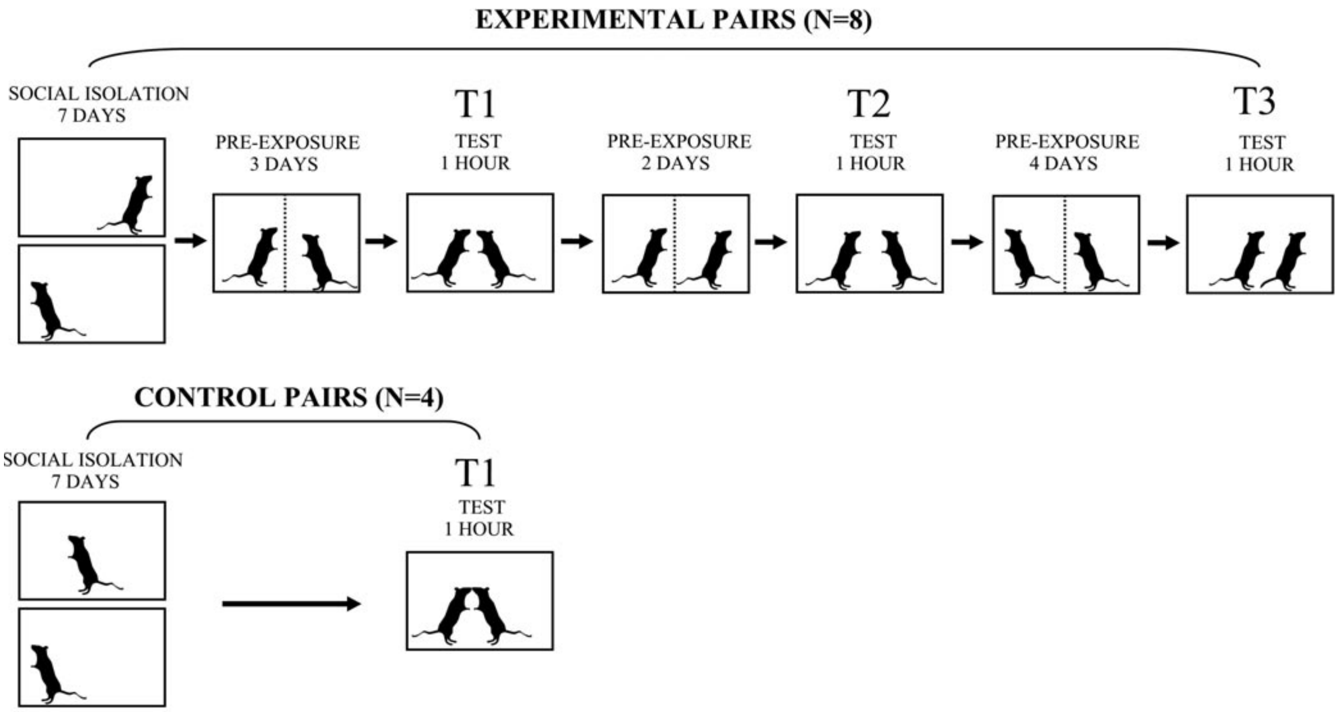
The tested sample comprised 24 wild adult (3–6 months old), male rats from F1–F3 generations of WWCPS stock (Warsaw Wild Captive Pisula Stryjek; Stryjek & Pisula 2008). All rats were unacquainted, unrelated (we avoided merging littermates into pairs) and of similar weights and ages, thus they were expected to be the most aggressive individuals. We chose males because aggression is generally higher than in females (Blanchard *et al* 1984, 1986; Miczek & de Boer 2005). The rationale behind choosing the most aggressive individuals (ie wild-type males) was that, if this method proves to be efficient when applied to highly aggressive and difficult-to-handle individuals, then it is likely to be useful with females and the more docile laboratory rats.

Research with laboratory-bred animals results in easy access and large sample sizes. However, wild rats are more difficult to gain access to, and often result in relatively small sample sizes (Parsons *et al* 2019). However, compared to most laboratory rodents, wild rodents provide a broader and more pronounced repertoire of behaviours; particularly aggressive behaviours (Barnett & Hocking 1981; Stryjek & Pisula 2008; Barnett 2009). However, some forms of behaviours — particularly ones being adaptive in laboratory conditions — are absent in wild rats. For instance, wild rats do not show submission by adopting the belly-up posture, which is crucial to diminish aggression between caged rats (Robitaille & Bovet 1976; Miczek & de Boer 2005). Since wild rats are more difficult to obtain and conduct research on, relatively smaller sample sizes are typical and expected (Byers *et al* 2017; Parsons *et al* 2019, 2020). Most bouts of aggressive behaviour occur between individuals of similar size (Jensen & Yngvesson 1998; Andersen *et al* 2000), therefore, weight differences between the tested individuals did not exceed 10% with weight range from 275 to 380 g.

Partition

Standard breeding cages equipped with lids and a built-in feed hopper were divided into two compartments using purpose-built wire-mesh partitions (Figure 1 [A]–[C]). To ensure that they were fully autoclavable, partitions were made of stainless steel. Partitions allowed the animals in separate compartments to come into contact (olfactory,

Figure 2



A schematic showing subsequent stages of the experiment. All tested rats were subjected to seven-day long social isolation. Rats from experimental groups (row 1) were tested three times and each measurement (T1, T2, T3) was preceded with a period of pre-exposure via wire-mesh partition. Rats from the control group (row 2) were tested directly after the period of social isolation.

auditory, visual, and, to some extent, tactile; Munoz & Blumstein 2012; Lecker *et al* 2015) with each other while eliminating the risk of a direct attack by either of the animals. The partitions were adjusted to the size of standard breeding cages (Eurostandard Type IV Tecniplast, Buguggiate, Italy; 612 × 435 × 216 mm [length × width × height]; floor area 2,065 cm^2). This design allowed us to leave the structure of the cage unaltered. The rats housed in the two compartments had access both to a feed hopper and to a water source (Figure 1[C]).

Procedure

Before the experiment, all animals had been housed in cages in groups of 4–5 individuals with dust-free softwood granules Tierwohl Super© (Allspan German Horse, Wismar, Germany) as bedding and *ad libitum* access to water and standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland). The day/night cycle was set at 12/12h, and temperature and humidity maintained at 21–23ºC and 45–60%, respectively.

During the first seven days of the trial, all rats were housed in social isolation with bedding made from dust-free softwood granules (Tierwohl Super©); they had *ad libitum* access to water and standard laboratory fodder (as above). Animals were randomly divided into three groups each comprising four pairs: experimental group 1 (Npairs = 4); 'Unsoiled' (tests conducted on new unsoiled bedding), experimental group 2 (Npairs = 4); 'Mixed' (tests conducted on mixed soiled bedding taken directly from both compartments of the divided cage inhabited for the previous 24 h by the tested pair of rats) and the control group (Npairs = 4) with tests conducted on new unsoiled bedding).

After the initial seven days of social isolation, pairs of rats from both experimental groups were placed into the breeding cages divided by the partition (Figure 2) for three days. The rats were then video-recorded for 60 min in a cage without the partition (Test 1; T1). This time-frame was selected because most significant aggressions occur shortly after the first contact. Some researchers (eg Koolhaas *et al* 2013) considered 10 min as sufficient to detect significant aggressive behaviours. However, we have learned that during the first minutes after direct exposure, wild animals usually spend the majority of time on exploratory behaviours and social interaction, while direct aggression usually occurs later. Thus, we elected to extend this period for more accurate results. We decided not to exceed 1 h of exposure for ethical reasons. Half of the rats from the experimental group were tested in a cage with unsoiled bedding, whereas the other half were tested in a cage with mixed bedding (soiled).

Following the observation period, rats were again separated by the partition for two days, after which the rats were observed again for 1 h (Test 2; T2). The rats were subsequently separated for four days and then observed for 1 h in a cage without a partition (Test 3; T3). The rats from the control group were tested in a shared cage with new bedding immediately after the seven-day social isolation (T1).

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To enable video-recording of observation sessions, a purpose-built case with a polycarbonate top (Figure 1[D]) was used instead of the standard wire-mesh cover. To ensure sufficient air flow, multiple holes had been cut in the polycarbonate top, which was placed 1 cm above the surface of the cage. The tests were conducted in the afternoons just before the lights in the vivarium were switched off. This allowed us to observe the rats at a time coinciding with their peak activity level (Stryjek *et al* 2013). The behaviour of each pair of rats was registered in total darkness in the experimental room with an infra-red video camera BCS-T460IR35 (NSS sp zoo, Warsaw, Poland) for 1 h. An additional source of IR illumination was provided via a hole in the side wall of the case (Figure 1[D]). The behaviour of the paired rats was closely monitored by a researcher so that excessively aggressive (prolonged periods of clinch attacks) behaviours could be easily spotted and the animals separated immediately. None of the pairings had to be ceased due to excessive fighting. For one of the control groups, the video camera malfunctioned meaning only the initial 30 min of interaction were able to be recorded and analysed.

Observed behaviours and hypotheses

We considered the following aggressive behaviours as most essential for our analyses (Blanchard *et al* 1986; Miczek & de Boer 2005; Barnett 2009; Plyusnina *et al* 2011; Koolhaas *et al* 2013): 'lateral threat displays' (often occurring jointly with pushing and ruffling); 'keep down' (where one rat holds the other down on the ground on its back); 'upright posture' (where both rats stand upright on their hind legs and 'hold' each other by their front legs); 'chase' (the pursuit of fleeing rat); 'clinch attack' (very rapid rolling, jumping, and biting both rats while being in close contact). Also, the following affiliative behaviours were identified: 'allogrooming'; 'crawling under' (where one rat crawls underneath the other); 'crawling over' (where one rat crawls over the other). We also measured 'social exploration' (sniffing at the other individual), the frequency and duration of 'grooming' and 'sleeping', as well as the number of 'rearings' (standing on hind legs extending head upwards).

We note that most of the above-described behaviours may occur during play fighting as well as aggression. However, this was not the case in our study because: (i) we examined adult individuals which are unlikely to engage in playful interactions; (ii) we provided a more 'hostile' situational context, which was likely to induce aggression; and (iii) video analysis showed that the observed rats attacked body parts typical to serious fighting, ie the rump and lower flanks (Himmler *et al* 2013). At this early stage, we were not concerned with the measurement of physiological parameters, as they can be invasive (Keay *et al* 2006), vary according to circadian rhythms (Sousa & Ziegler 1998), and can be collected in the future if the partition-approach shows merit. Behavioural data coding was performed by a researcher who was blind to the treatment.

We hypothesised that dividing rats with a purpose-built wire-mesh partition (Figure 1 [A]–[C]) for periods of 1, 3, and 7 days will result in a significant reduction of all measured aggressive behaviours and social exploration. We also assumed that using mixed bedding with obvious olfactory traces (urine, sebum, dander, porphyrin, faeces) of introduced animals will intensify territorial and aggressive behaviours (Adams 1976; Koolhaas *et al* 2013).

Statistical analysis

Given the constraints of working with wild rats (limited access to wild animals, ethical considerations, an unknown estimate of mean and standard deviation in the population; Festing 2006), we elected to use eight animals (ie four pairs) of rats in each group. We justify this approach because this number is already reasonable for wild rat studies (Byers *et al* 2017; Parsons *et al* 2019) and averaging results from paired animals improves measurement precision (Haseman & Hogan 1975). To account for non-normal distributions and a relatively small sample size as compared to laboratory studies with domesticated rodents, and to ensure we did not falsely reject our null hypothesis, we decided *a priori* to use non-parametric tests (Tacha *et al* 1982). Mann Whitney U tests (SPSS v26, Armonk, NY, USA) were used for pairwise comparisons between the groups. Friedman's rank test and Wilcoxon signed-rank test were used to compare consecutive trials and intervals. Pairs, but not single animals, were considered to be units of replication.

Results

Twenty-eight videos were scored for each behaviour. Measurement sessions were marked as follows: T1 (the first measurement; in the control group the measurement was taken at the moment of placing a pair of rats directly in a shared cage with new bedding; in the experimental groups the measurement was taken at the moment of placing a pair of rats in a shared cage after the seven-day social isolation period); T2 (measurement in the Unsoiled and Mixed groups after three days in a cage divided by a partition); T3 (measurement in the Unsoiled and Mixed groups after seven days of staying in a cage divided by a partition). The T1 measurement for the control group was compared with all the measurements taken for the 'Unsoiled' and 'Mixed' groups (T1, T2 and T3).

Since no differences were observed between the Unsoiled and Mixed experimental groups (Mann-Whitney U test; $P > 0.1$), aggregated results from both experimental groups were used in the following analysis.

Cross-sectional analysis

Aggressive behaviours

No differences were observed between the subsequent measurement sessions conducted in the experimental group (T1, T2, T3) with respect to all aggressive behaviours analysed (Friedman's rank test; $P > 0.1$).

Pre-exposure decreased the number of 'lateral threats' and 'upright postures' in all experimental sessions and a decrease of 'keeping down' in T1 and T2 sessions; see Figure 3.

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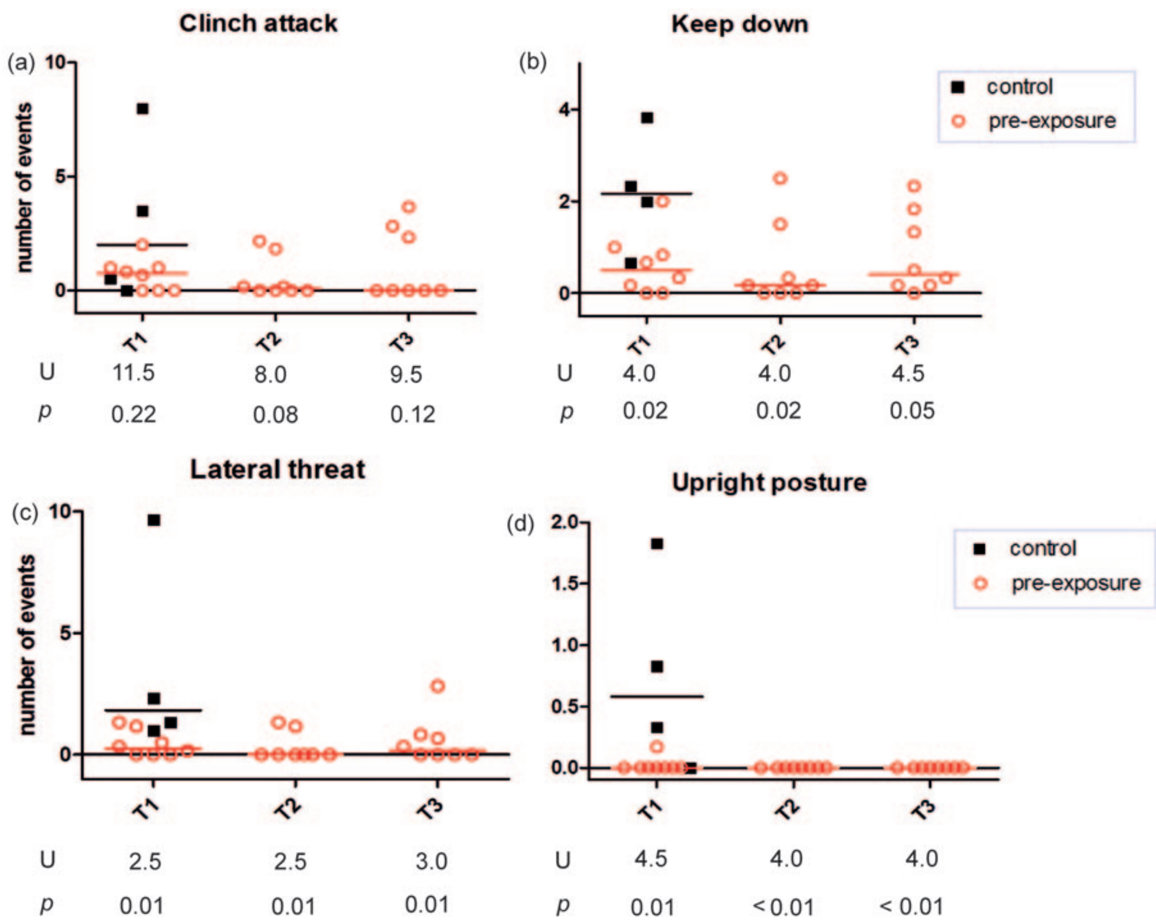
Figure 3



Comparisons of selected aggressive behaviours between caged rats that were subjected to pre-exposure and controlled non-treated individuals. From top left clockwise, numbers of events of: clinch attacks, keep down behaviours, upright postures, and lateral threats (average values for 10-min intervals). The bars represent median values. Below each graph: Mann Whitney *U* test results (one-tailed) for the control group compared with three measurements of the experimental group. The major significant differences in the case of pre-exposed rats were: a decrease in displays of lateral threats, upright postures, and keeping down an opponent.

Affiliative behaviours

No differences were observed between the subsequent measurement sessions conducted in the experimental group (T1, T2, T3) with respect to affiliative behaviours analysed (Friedman test; $P > 0.05$). We also found no difference between the control and experimental groups in displays of all measured affiliative behaviours (Mann-Whitney *U* test; $P > 0.14$).

Other behaviours

No differences were observed between the subsequent measurement sessions (T1, T2, T3) with respect to social exploration (Friedman's rank test; $P > 0.7$).

We found that the rats that were pre-exposed prior to being merged into one cage displayed less social exploration (median = 3.5) compared to the control group (median = 11.33) and slept for a significantly longer time (median = 25.19) as compared to the control group (median = 1.14) (see Figure 4). They also had more sleeping bouts (median = 3.25) as compared to the control group (median = 0.25) and expressed less rearing (median = 12.17) as compared to the control group (median = 28.67).

Median values and quartile deviations for each behaviour in each measurement are presented in Table 1

Analysis of behavioural dynamics in session T1

The following calculations are based on a comparison of data obtained for two 30-min intervals (first and second half of the T1 session, ie the first direct pairing of rats). The analysis was carried out separately for the control group and the experimental groups using one-tailed Wilcoxon signed-ranks tests.

No statistically significant differences were observed between the intervals with respect to all behaviours in the control group. In the case of pre-exposed rats (experimental group), we found significant decrease between the intervals in the number of 'clinch attacks' ($Z = -1.84$; $P = 0.033$), 'keep downs' ($Z = -1.99$; $P = 0.023$), 'lateral threats' ($Z = -2.03$; $P = 0.021$),

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'crawling over' (Z = –1.70; P = 0.045), 'crawling under' (Z = –2.07; P = 0.019), bouts of 'social exploration' (Z = –2.52; P = 0.006), 'grooming' time and frequency (Z = –1.82; P = 0.034 and Z = –2.52; P = 0.006), the number of 'rearings' (Z = –2.52; P = 0.006), and an increase of frequency and time of 'sleeping' (Z = –2.20; P = 0.014 and Z = –2.20; P = 0.014), respectively.

Figure 5 presents significant differences in selected behaviours.

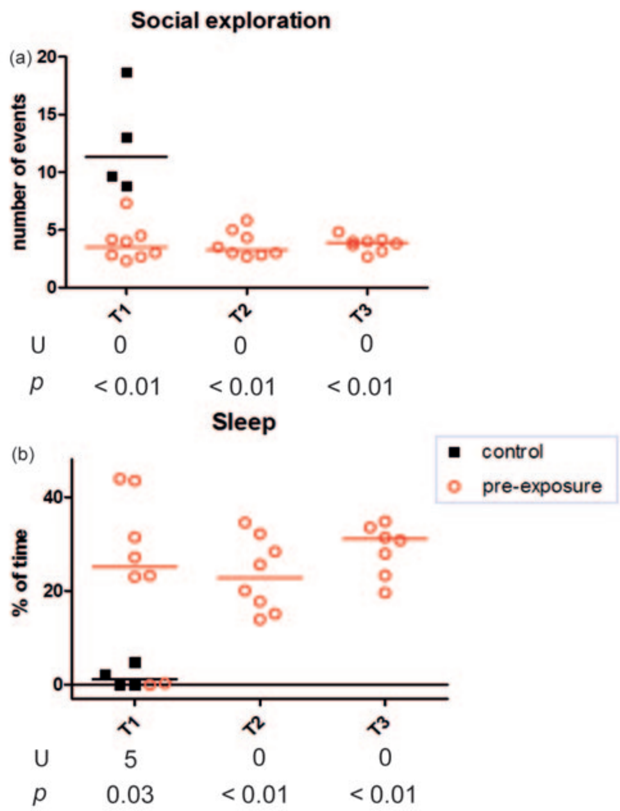
Discussion

Species such as elephants, pigs, horses and a variety of laboratory species may need to be introduced for social or breeding purposes (Sanchez *et al* 1993; Forde & Marchant-Forde 2005; Tresz & Wright 2006; Marchant-Hartmann *et al* 2009; Fawcett & Rose 2012; Tallent *et al* 2018). Though these introductions and pairings are essential for breeding or experimental purposes, in addition to loss of experimental data and research quality (Pisula *et al* 2006), they may cause stress, suffering, and even death. At least among rodents, the partition we deployed shows promise as a welfare-friendly approach to limit aggression caused by introducing new individuals into existing groups.

Overall, wild-type rats subjected to pre-exposure by partition exhibited significantly fewer bouts of aggressive behaviour. Among the five focal behaviours, three ('lateral threats', 'upright postures' and 'keep downs') were statistically significant. We believe the reason for our findings relates to the exchange of multimodal sensory information across the permeable partition. Rats from the experimental group showed lower levels of social exploration, which supports the view that they exchanged social information during the pre-exposure stage. Further, the pre-exposed rats slept more than controls. Although an increase in sleep intensity is sometimes attributed to a high level of stress and exhaustion (Cespuglio *et al* 1995; Meerlo *et al* 1997), this seems unlikely in our trials, as rats in the control group remained vigilant and active through the whole experimental session.

The three subsequent temporal measurements (after 3, 5 and 9 days of pre-exposure) showed no statistically significant differences in the level of aggressive or affiliative behaviours or social exploration. This may suggest that a three-day pre-exposure is enough to significantly reduce the number of bouts of aggressive behaviour; at the same time, a prolonged use of this method does not lead to further reduced aggression. A comparison of the measurement results for the rats which were paired in cages with new, unsoiled bedding with the results obtained for the rats paired on mixed, soiled bedding (the bedding used for several days in both compartments) yielded no significant differences, which is consistent with results from studies with wild rats conducted by Alberts and Galef (1973). In our study, the odour intensity of rats' own and alien territory (via the scent of the soiled bedding) did not prove to be a reliable predictor of aggression. It could possibly be explained by a cancellation effect of aggression being reduced by the scent of nesting material and intensified by the scent of the bedding containing urine and faeces (Van Loo *et al* 2000). Therefore, it seems advisable to use new bedding, both for hygienic reasons and to enhance the ease of the procedure.

Figure 4



Comparisons of selected non-aggressive and non-affiliative behaviours between caged rats that were subjected to treatment (pre-exposure) and control (non-pre-exposed individuals). Showing (a) number of events of social exploration (average values for 10-min intervals) and (b) proportion of time spent sleeping. The bars represent median values. Lower: Mann Whitney *U* test results (one-tailed) for the control group compared with three measurements of the experimental group. The major significant differences in preexposed rats were a decrease in social exploration and an increase of sleeping time.

It is worth noting that subjecting rats to pre-exposure did not completely eliminate aggressive behaviours. Although the mean number of clinch attacks (the cause of most injuries) dropped significantly between the first and the second half of the T1 session (the first direct contact between the animals), the overall decrease was not statistically significant in either session (see Figure 3). The fact that wild rats' base level of aggression is much higher than that of lab rats (Barnett & Hocking 1981; Stryjek & Pisula 2008; Barnett 2009), however, supports our hypothesis that pre-exposure may be more efficient in laboratory rats because their basal level is much lower at the start and, hence, probably easier to be inhibited.

To conclude, if the partition we used in our study works with the most aggressive Norway rats (ie, wild males of equal size),

Table 1 Descriptive data on the different types of behaviour by group. Medians and quartile deviations (QD) are provided for ten-minute intervals.

					T1		T2		T3	
Group	**Category**	**Behaviour**	**Units**	**Npairs**	**Median**	**QD**	**Median**	**QD**	**Median**	**QD**
Experimental	Aggressive	Lateral threat	Number	8	0.25	0.50	0.00	0.44	0.17	0.40
		Keep down	Number	8	0.50	0.46	0.17	0.60	0.42	0.77
		Upright posture	Number	8	0.00	0.00	0.00	0.00	0.00	0.00
		Clinch attack	Number	8	0.75	0.50	0.08	0.71	0.00	1.35
		Chase	Number	8	0.00	0.21	0.00	0.00	0.00	0.00
	Affiliative	Allogrooming	Number	8	0.75	0.60	1.25	0.77	0.75	0.23
		Crawling over	Number	8	0.66	0.46	0.67	0.42	0.25	0.58
		Crawling under	Number	8	0.17	0.15	0.00	0.00	0.00	0.08
	Other	Social exploration	Number	8	3.50	0.85	3.25	0.98	3.92	0.42
		Grooming	Number	8	12.67	3.04	14.33	2.67	15.58	1.71
		Grooming	Duration (%)*	8	11.03	4.20	19.53	5.20	13.93	4.15
		Sleep	Number	8	3.25	1.35	3.58	1.23	5.08	1.29
		Sleep	Duration (%)*	8	25.19	17.26	22.83	7.74	31.06	5.03
		Rearing	Number	8	12.17	6.23	9.00	2.67	10.83	1.73
Control	Aggressive	Lateral threat	Number	4	1.83	3.38				
		Keep down	Number	4	2.17	1.23				
		Upright posture	Number	4	0.58	0.75				
		Clinch attack	Number	4	2.00	3.38				
		Chase	Number	4	0.08	0.21				
	Affiliative	Allogrooming	Number	4	0.33	0.90				
		Crawling over	Number	4	1.33	0.77				
		Crawling under	Number	4	0.00	0.13				
	Other	Social exploration	Number	4	11.33	4.10				
		Grooming	Number	4	22.83	7.85				
		Grooming	Duration (%)*	4	15.06	4.35				
		Sleep	Number	4	0.25	1.00				
		Sleep	Duration (%)*	4	1.14	2.10				
		Rearing	Number	4	28.67	8.44				

* For an individual rat.

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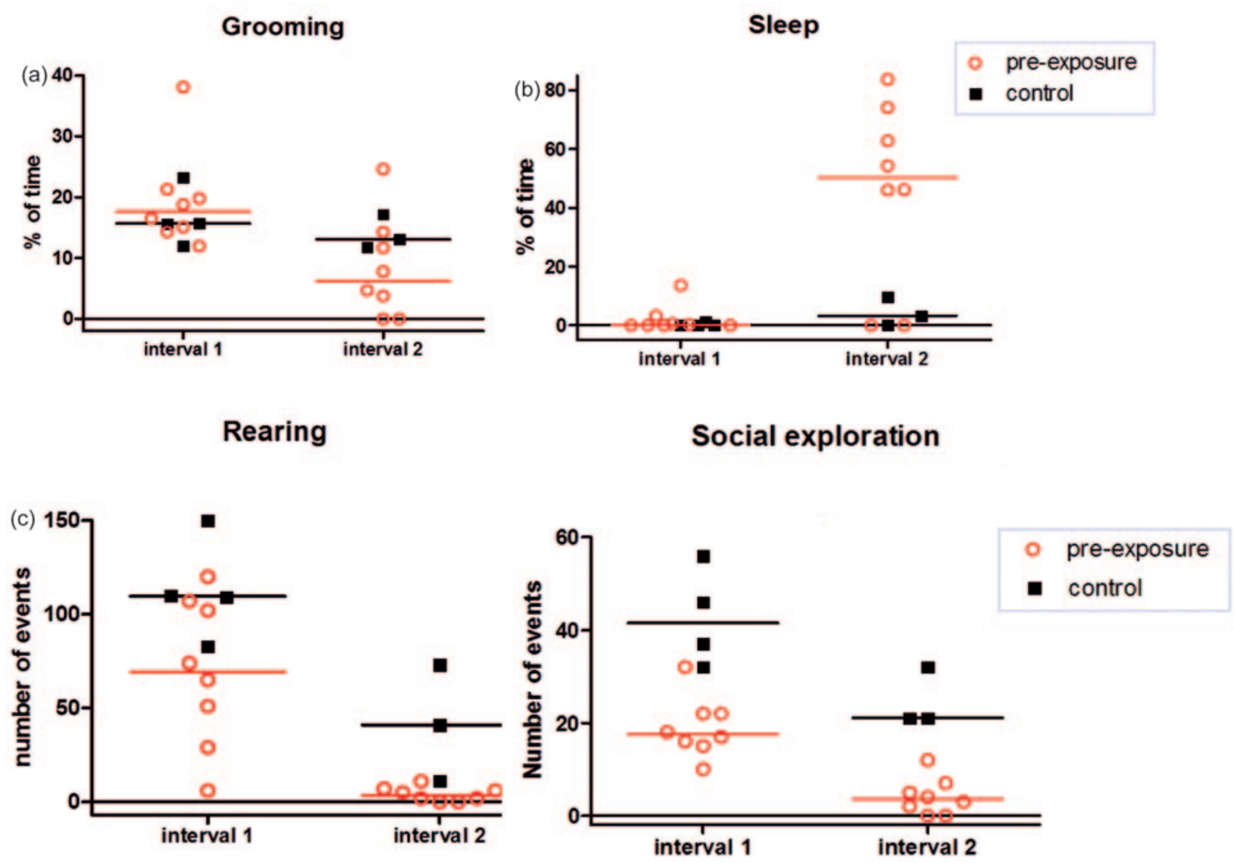
Figure 5



Selected measured non-aggressive but anxiety-related behaviours during T1 session for two subsequent 30-min intervals for caged rats that were subjected to pre-exposure and controlled non-treated individuals. From top left clockwise: percent of time spent grooming, percent of time spent sleeping, number of events of social exploration, and number of rearings. The bars represent median values. In the case of pre-exposed rats all variables differed significantly between the intervals (significant decrease in grooming, rearings, and social exploration, and increase in the time spent sleeping). No significant differences in the control group were found.

then it is reasonable to assume it might work with less aggressive individuals such as wild females and male and female laboratory rats. To further the aims of animal welfare, further research in this field may follow from this study.

Future studies should also involve analysing the behaviour of rats during the pre-exposure period (ie, while separated by the partition). This approach would allow more insight into the process of familiarisation and establishment of hierarchy, and potentially provide a means for predicting overt fighting in rats separated by the partition to indicate the most aggressive individuals/pairs. Such a safe (ie, not involving direct contact) protocol would prevent merging animals that would be the most likely to cause harm to each other.

It is worth noting that keeping animals separated by a partition is stressful itself, as similar procedures are sometimes used to study chronic psychosocial stress in animals (CPS); (Pryce & Fuchs 2017). Additionally, studies conducted on female mice during post-operative recovery suggest that mice exposed via partition to conspecifics may be more distressed than mice experiencing direct contact (Van Loo *et al* 2007). The stress is possibly because of the accumulation of pheromonal stimuli (Kudryavtseva 1991). While we should keep an open mind to this possibility, our results show a clear decrease in aggressive behaviours. To shed more light on the efficiency of the described method it is advised that additional metrics be used in future studies, including physiological indices, eg heart rate (Sgoifo *et al* 1999) and corticosteroids level (Keay *et al* 2006). Similar approaches may be attempted with other social species, particularly those using scents or multimodal inspection to assess intruders.

Animal welfare implications

Pairings of unfamiliar rats may result in elevated stress and aggression. However, using a permeable barrier that maintains temporary separation between merged animals reduces this risk while allowing animals to become familiar with one another. Ultimately, this could provide a humane mechanism to introduce new individuals into pre-existing colonies.

Declaration of interest

The authors declare that there is no conflict of interest regarding the publication of this article. The funder of the work played no role in the study design; collection, analysis and interpretation of data; writing of the paper; and the decision to submit to *Animal Welfare*.

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